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MINIREVIEW

Cell Surface Structures of Archaea▽

Sandy Y. M. Ng,[1] Behnam Zolghadr,[2] Arnold J. M. Driessen,[2] Sonja-Verena Albers,[2] and Ken F. Jarrell[1]*

Department of Microbiology and Immunology, Queen's University, Kingston, Ontario, Canada K7L 3N6,[1] and Department of Molecular Microbiology, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands[2]

Prokaryotes possess various kinds of cell surface organelles serving versatile biological roles depending on the environmental niche of the organism. The formation of these structures involves fascinating machineries, as not only do the protein components need to travel across the cytoplasmic membrane like all secreted proteins, but they also need to do so in a precisely coordinated manner for proper assembly. Most commonly found on the surface of bacteria are flagella used for swimming (47); the type III secretion injectisome (needle structure) (21), which is used to deliver effector molecules from pathogenic organisms into host cells; and a wide variety of thinner organelles that fall under the broad designation of pili (13, 33, 58, 64, 69, 78). Different classes of these structures (type I pili, type IV pili, sex pili, etc.) which differ significantly in their structure, assembly, and function have been identified. Their many roles include adhesion, twitching (or surface) motility, and delivery of DNA and toxins, as well as functioning as electrically conductive "nanowires." Other, less commonly studied appendages have also been reported, such as spinae (9).

Archaea, representing the third domain of life, have been isolated from the most extreme environments known to harbor life (15). They have been cultivated from environments that mark the upper limits of life in regard to temperature, pH, and salt concentration. Furthermore, molecular techniques have indicated that archaea are common in a variety of less extreme niches, such as ocean waters, freshwater sediments, and soil (20, 23, 66). Evidence points to a broader and more significant role in the ecosystem for archaea than previously believed (12, 14, 49).

Both directed studies of surface structures in archaea, as well as electron microscopic observations of a variety of diverse archaea, have indicated a number of different types of surface structures. Some, like the archaeal flagella and pili, appear at first glance to be similar to their bacterial counterparts but possess significant differences and archaeal twists. Other structures, such as cannulae and hami, appear to be unique to the archaeal domain. Still other structures, such as the bindosome, are predicted to exist from genetic and biochemical data although direct electron microscopic evidence is, as yet, lacking. Studies on archaeal extracellular structures have enriched our understanding of a variety of topics, including protein export, posttranslational modifications, assembly mechanisms, and metabolism. They have also provided unique examples of adaptations to their environments not observed in bacteria. The capacity of some archaea to assemble these structures in extreme conditions that bacterial structures often fail to withstand is of great interest from both fundamental and applied viewpoints.

FLAGELLA

The best studied of all archaeal surface structures is the archaeal flagellum. Flagella are widely distributed throughout all the major subdivisions of cultivatable archaea from both *Crenarchaeota* and *Euryarchaeota* (38, 55), including halophiles, haloalkaliphiles, methanogens (Fig. 1), hyperthermophiles, and thermoacidophiles (see Fig. 7B), with at least some studies on flagella in representatives of all these groups (11, 17, 28, 30, 32, 41, 54, 61, 63, 76).

Generally, flagellated archaea have a single major identified genetic locus involved in flagellation (55). This locus usually begins with multiple structural flagellin genes (*flaA* and/or *flaB* genes), followed by *fla*-associated genes *flaC* to *-J* or some subset of these genes (an exception is the single flagellin gene found in *Sulfolobus* species [(3]). Most of the complete *fla*-associated gene sets are found in *Euryarcheota* while the *Crenarchaeota* have only a subset of these genes. The preflagellin peptidase gene, *flaK/pibD*, which encodes an essential signal peptidase required for flagellin processing, is usually located elsewhere on the chromosome. The functions of *flaC* to *-J* are mainly unknown, although FlaI contains ATPase activity (1) and, due to its homology to type IV pilus system ATPases (10), is believed to be critical for assembly. Various genetic studies, especially with *Methanococcus* and *Halobacterium*, have indicated that (probably) all of the *flaC* to *-J* gene products are necessary for proper assembly and function of the flagella (17, 61).

While performing the same function as bacterial flagella, the flagella of archaea are thought to be a unique motility apparatus (80). Evidence accumulated over the years has led to the proposal that archaeal flagella are more similar to bacterial type IV pili than to bacterial flagella (10, 29, 55, 81). At the gross morphological level, archaeal flagella are rotating structures with a filament, as seen in bacterial flagella (8, 22, 43). However, archaea do not possess any homologues of genes found in bacteria that are involved with bacterial flagellum structure or assembly (27, 59). On the other hand, archaeal

* Corresponding author. Mailing address: Department of Microbiology and Immunology, Queen's University, Kingston, Ontario, Canada K7L 3N6. Phone: (613) 533-2456. Fax: (613) 533-6796. E-mail: jarrellk@queensu.ca.

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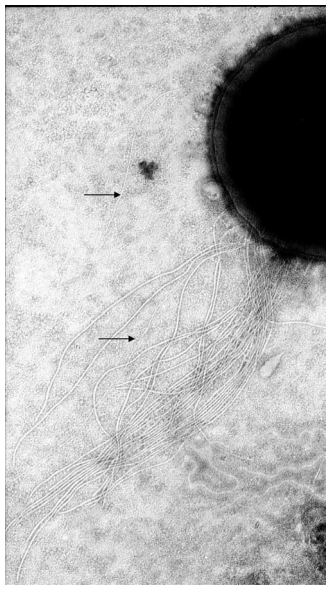

FIG. 1. *Methanococcus maripaludis* showing presence of flagella and thinner pili (arrows). Flagella are approximately 12 nm in diameter, and pili are about 6 nm. The sample was negatively stained with 2% phosphotungstic acid, pH 7.

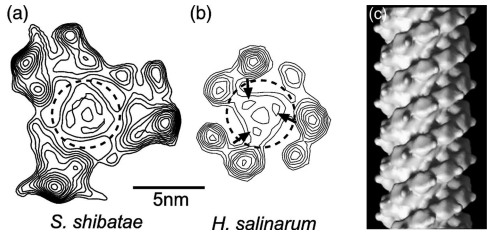

FIG. 2. Comparison of the flagellar filament of *S. shibatae* to that of *H. salinarum*. (a and b) Contoured cross-sections of the three-dimensional reconstructions of *S. shibatae* (a) and *H. salinarum* (b) are shown side by side to scale. The inner core domain (broken circle), which was modeled in *H. salinarum* as α-helices (arrows), is similar in size and shape in both archaea. The difference in diameters (14 nm and 11 nm, respectively) stems from the dimensions of the outer domains. In the helically arranged structure there appear to be additional interactions between the outer domains in *S. shibatae*. (c) Three-dimensional structure of the *S. shibatae* flagellar filament. (Reprinted from reference 18 with permission of the publisher.)

flagellins have conserved amino acid sequences at their N termini, both in the mature proteins and in their class III signal peptides, which are similar to type IV pilins (29, 75). Like type IV pilins, archaeal flagellins are made as preproteins with short, atypical signal peptides, which are cleaved by a specific prepilin peptidase-like enzyme that is conserved in both the type IV pilus and archaeal flagellum systems (3, 6, 7, 74). Much work has been done on the specific peptidase in *Methanococcus* and *Sulfolobus*. Mutants lacking this enzyme cannot make flagella and their flagellins retain the signal peptide, indicating that processing of the signal peptide is a critical step in the assembly of the flagellum structure (6). Studies of the critical amino acids of the substrate flagellins needed for processing indicate the importance of the highly conserved −1 (relative to the cleavage site) glycine as well as other amino acid positions in the signal peptide, although the *Sulfolobus* PibD enzyme appeared to be more flexible in its requirements (6, 74). This has been interpreted to mean that PibD perhaps has less stringent requirements than other processing peptidases, which is supported by the additional substrates recognized by *Sulfolobus solfataricus* PibD, i.e., a variety of sugar binding proteins (3, 75). Site-directed mutagenesis of the FlaK/PibD enzymes has indicated the critical importance of two aspartic acid residues for activity and has demonstrated that the archaeal prepilin peptidase-like enzymes are indeed members of the prepilin peptidase enzyme class (6, 74).

Further evidence that supports a relationship between archaeal flagellum and type IV pilus systems is the conservation of two other proteins. Critical for assembly of both structures is an ATPase (10), i.e., PilT in the pilus assembly system (*Pseudomonas* nomenclature is used) and FlaI in the flagellum system, as well as a conserved membrane protein, i.e., PilC and FlaJ (62). A final piece of evidence is that the architecture of type IV pili and archaeal flagella is also shared and is different from that of bacterial flagella (19, 81). Recently, it has been shown that the flagella of two phylogenetically distant members of the *Archaea*, the extremely halophilic *Halobacterium salinarum* and thermoacidiphilic *Sulfolobus shibatae*, share a common subunit packing which is unlike that of bacterial flagella (18). The results indicate a symmetry for flagella of this domain that separates them from their bacterial counterparts. Critical in this analysis is the lack of a central channel in archaeal flagella (Fig. 2) (18, 19), a feature essential for bacterial flagellum assembly. The majority of the external portion of the bacterial flagellum (rod, hook, and filament) is assembled by means of a type III secretion system located at the base of the MS ring (46). Through this complex, substrates which lack cleaved signal peptides are pushed through the hollow structure itself so that the subunits assemble at the distal tip furthest from the cell body. Clearly, with archaeal flagella lacking a central channel large enough to accommodate structural proteins, the assembly mechanism cannot be as in bacterial flagella. Based on the similarities to type IV pili already mentioned, it was proposed that assembly of archaeal flagella might occur with subunits added at the base (Fig. 3) (39).

Much work has been done recently on the glycosylation of the archaeal flagellins. Unlike the case for bacterial flagellins, which have, on occasion, been shown to bear O-linked glycans (45), in both halobacteria and methanococci the attached glycan is N linked. In *Methanococcus voltae* the glycan is a trisaccharide (83). Following a model for N-linked glycosylation based on similar systems in bacteria and eukaryotes, it was expected that three glycosyltransferases would be needed to assemble the *M. voltae* trisaccharide glycan on the lipid carrier, dolichol phosphate. Once assembled, the glycan is thought to be flipped to the external side of the cytoplasmic membrane by a flippase and then attached to the flagellin at an asparagine residue within a conserved sequence of Asn-X-Ser/Thr. This final step is catalyzed by an oligosaccharide transferase. In *M. voltae*, the glycosyltransferase catalyzing the transfer of the final sugar to the glycan was identified (AglA), as was the oligosaccha-

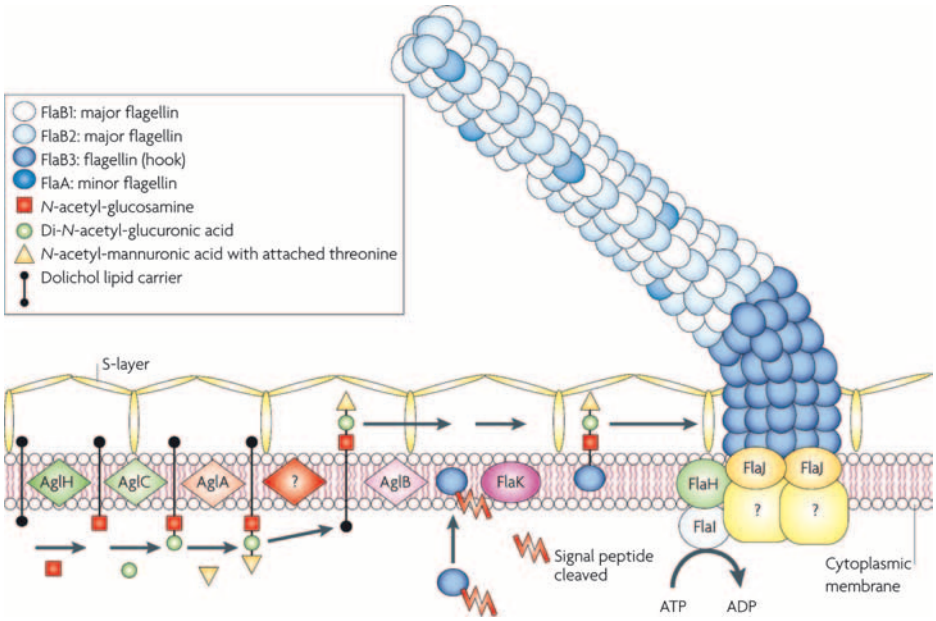

FIG. 3. Proposed model for assembly of archaeal flagella using *Methanococcus voltae* as a model. A three-sugar glycan is assembled via the activity of glycosyltransferases (AglH, AglC, and AglA) onto a dolichol phosphate lipid carrier, flipped to the external face of the cytoplasmic membrane via an unknown mechanism, and then transferred to the flagellins via an oligosaccharyltransferase (AglB). Upon removal of the signal peptide by FlaK, the processed flagellins are incorporated into the base of the structure, likely with the aid of the ATPase FlaI. (Reprinted from reference 37a.)

ride transferase (AglB), which was homologous to PglB of bacteria and the Stt3 subunit of yeast (16). More recently, the first glycosyltransferase (AglH), which transfers *N*-acetylglucosamine to dolichol, was identified by the ability of the *M. voltae* gene to complement a conditional lethal mutation in the equivalent yeast gene product Alg7 (71). Attempts to delete the methanogen gene directly repeatedly failed, indicating its possible essential nature. In addition, a glycosyltransferase (AglC) needed for the transfer of the second sugar was also recently identified (B. Chaban, S. M. Logan, and K. F. Jarrell, unpublished data). To date, the expected flippase has not been reported. Interestingly, mutants missing or with a truncated glycan on the flagellins were either nonflagellated or poorly flagellated, indicating a critical role for glycosylation in flagellum assembly (16). Glycosylation of flagellins appears to be a widespread trait in archaea (38), including many that thrive in extreme environments, suggesting that another possible role for glycosylation may be to aid in protein stability in coping with such harsh niches (25).

Many archaea are known to be chemotactic and/or phototactic (38, 60, 68). The ability of archaea to respond to changes in their environment is through a system very similar to the chemotaxis system found in bacteria. Indeed, a complete set of *che* genes can be found in many flagellated archaea. In addition, archaeal flagella are known to be able to switch their rotation (53, 59). In bacteria, the connecting point of chemotaxis and flagella occurs when phosphorylated CheY binds to the switch component FliM of the flagella (5, 77). However, while *cheY* genes are readily found in the genomes of flagellated archaea, the corresponding interacting switch protein has never been reported, and the site of interaction of the chemotaxis system and the flagella remains a mystery.

Flagella of bacteria perform many roles in addition to their key role in swimming in liquid environments. Flagella can also be used by certain bacteria to swarm across solid surfaces (34). Sometimes, this is performed even by a flagellar system (lateral flagella) that is separate and independent from the one responsible for swimming (48, 50). Furthermore, it was recently shown that bacterial flagella can sense wetness, as well as be involved in adhesion and biofilm formation (85). While much less studied, it seems that archaeal flagella may also play roles in addition to just being a swimming organelle. For example, they have been shown to be essential for swarming motility on plates in *S. solfataricus* (76). In addition, in *Pyrococcus*, flagella appear to act as cables connecting cells, perhaps as an initial prerequisite for genetic transfer, and in adhesion to abiotic surfaces (54). Recently, it has been shown that interactions between *Pyrococcus furiosus* and *Methanopyrus kandleri* can occur through flagella as well as cell-to-cell contact, resulting in the formation of a structured bispecies biofilm (70).

Two archaea, *Methanopyrus kandleri* and *Pyrobaculum aerophilum*, have been described as being motile and flagellated, but typical archaeal flagellin genes could not be identified in the sequenced genomes. In *M. kandleri*, where polar tufts of flagella have been reported (44), open reading frames encoding extremely short, flagellin-like proteins and clustering near *flaHIJ* homologues have been identified. If these are authentic flagellin-encoding genes, the resulting flagellins would be much shorter (approximately 80 amino acids in length) than typical flagellins. In *P. aerophilum*, bundles of flagellum-like filaments, with the typical diameter of 10 nm often reported for

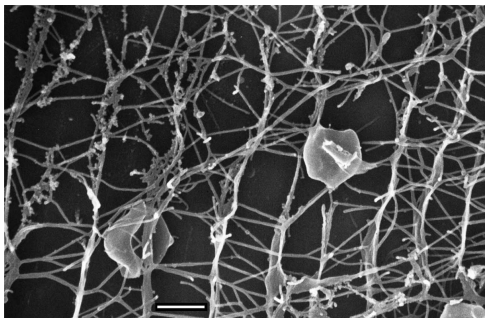

FIG. 4. Part of a network of *Pyrodictium* cells and cannulae, with tubules in a regular array. Scanning electron microscopy was used. Bar, 1 μm. (Reprinted from reference 65 with permission of the publisher.)

archaeal flagella, were observed (84), but flagellin and *fla*-associated genes have not been identified.

CANNULAE

Members of the genus *Pyrodictium* have been isolated from various hydrothermal marine environments all over the world (72, 73), with growth temperatures ranging from 80 to up to 110°C. A unique feature of this group of organisms is that cells grow in a network of tubules termed cannulae (Fig. 4), which connect the cells with each other. Cannulae are hollow tubes with an outside diameter of 25 nm, and they appear empty when cross-fractured or thin-sectioned (65). They consist of at least three different, but homologous, glycoprotein subunits with identical N termini but with different molecular masses of 20, 22, and 24 kDa (57, 65). The structure of the cannulae is highly resistant to heat as well as denaturing agents, as no morphological changes were observed after 60 min of incubation at 140°C or 10 min at 100°C and 2% sodium dodecyl sulfate (65). During the analysis of cell division of *Pyrodictium abyssi* TAG11, it became evident that two newly formed daughter cells always stay connected with the growing cannulae (36), leading to a dense network of cells and cannulae at the end of the cultivation. The final length of the cannulae varied between 30 and 150 μm with an elongation rate of 1.0 to 1.5 μm/min, which is significantly higher than the elongation rate for bacterial flagella, e.g., *Salmonella* (0.16 μm/min in in vitro measurements) (37).

Three-dimensional reconstruction of cannula-cell connections showed that cannulae clearly enter the periplasmic space but not the cytoplasm, while other cannulae barely touched the S-layer (57). This was the first evidence that cannulae function as an intercellular connection of periplasmic spaces between different cells. Interestingly, certain marine bacteria produce an unusual tubular surface structure, termed spinae, that is capable of forming a broadly similar network. Approximately 11 nm in diameter and apparently open tubes, these spinae have been demonstrated to connect cells over distances of several micrometers. Ultrathin sections show that the inner and outer membranes are often joined at the site of spina insertion, leading to the hypothesis that spinae might allow exchange of signals between the connected cells (9). However, the function of the cannula network is still unclear. It might act to anchor cells to each other or as a means of communication for the exchange of either nutrients or even genetic material. Currently, little is known about the mechanisms and kinetics of diffusion of biological materials within a cannula tubule.

HAMI

Another fascinating, recently reported archaeal cell surface appendage is the archaeal "hamus" (52). This structure represents a novel filamentous cell appendage of unexpectedly high complexity. Archaeal cells bearing these structures are found in macroscopically visible string-of-pearls-like arrangements among bacterial filaments, mainly *Thiothrix* (SM) or IMB1 proteobacterium (IM) growing in cold (~10°C), sulfidic springs (67). The individual pearl can reach a diameter of up to 8 mm. Scanning electron microscopy and fluorescent in situ hybridization revealed that the filamentous bacteria form the outer, whitish part of the pearl, as well as the connecting threads, with each interior of the pearl dominated by up to 10^7 euryarchaeal SM1 cells. The archaeal cells are coccoids approximately 0.6 μm in diameter. Attached to each archaeal cell are approximately 100 filamentous hami, each 1 to 3 μm in length and 7 to 8 nm in diameter (Fig. 5a) (52). The hamus filament has a helical basic structure, with three prickles (each 4 nm in diameter) emanating from the filament at periodic distances (46 nm). At the distal end, a tripartite, barbed grappling hook, 60 nm in diameter, was identified (Fig. 5b and c). The hamus is composed mainly of a 120-kDa protein. Chemical testing revealed that the hami remain stable over broad temperature and pH ranges (0 to 70°C; pH 0.5 to 11.5). As such, these filamentous structures mediate strong cellular adhesion for the archaeal cells to surfaces of different chemical compositions.

Recently, in a sulfidic spring near Regensburg, Germany, slime-like, milky drops consisting almost entirely of the SM1 euryarchaeon were harvested (35). This organization differs from the previously described string-of-pearls structures in several remarkable ways. While the archaeon-to-bacterium ratio in the string-of-pearls community is approximately 1:1, the SM1 biofilm is dominated by archaeal cells, representing the first archaeal monospecies biofilm in nature. Confocal laser scanning microscopy revealed a constant and regular three-dimensional arrangement of the archaeal cells, with cells being approximately 4 μm apart. The regular distance is speculated to be caused by the hami of neighboring cells, which have an average length of 2 μm. The hamus also forms the main protein component of the extracellular polymeric substances, thereby contributing to the biofilm structure. Several gaps, resembling the typical water channels in bacterial biofilms, were also observed. Henneberger and colleagues (35) proposed that the hami function in surface attachment and biofilm initiation, much like flagella and pili can in bacterial biofilm formation.

PILI

Weiss reported as early as 1973 that *Sulfolobus* cells taken freshly from a hot spring are attached to sulfur particles by numerous 5-nm-wide pili (87). Pili have also been observed in many other archaea (24, 42, 51), but a detailed analysis of the identity of the subunits or their assembly mechanisms has not

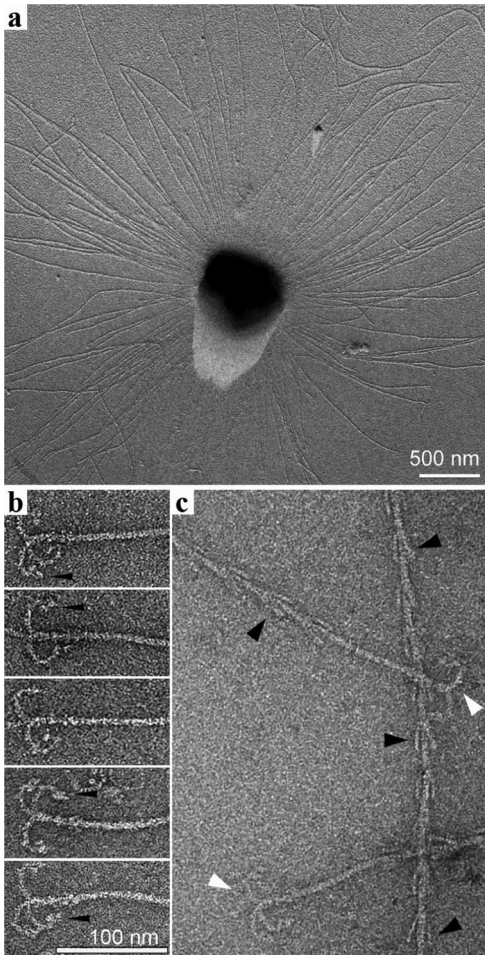

FIG. 5. (a) Electron micrograph of a platinum-shadowed SM1 euryarchaeal coccus. About 100 hami emanate radially from the cell surface. (b) Electron micrographs of grappling hooks located at the distal ends of the hami. Arrowheads indicate locations of barbs. (c) Electron micrograph of high-level-structured SM1 hami. The hami show prickles and grappling hooks. (Reprinted from reference 52 with permission of the publisher.)

been reported yet. The FlaFind program (75) predicted a variety of possible pilin-like proteins in sequenced archaeal genomes. The genes of many of these predicted proteins are located in operons together with genes encoding homologues of TadA and TadC. This suggests that these operons encode proteins that assemble pili or pilus-like structures. The FlaFind analysis also identified a group of putative pilins with a DUF361 domain which have a different cleavage site consensus sequence than the other prepilins that are processed by FlaK/PibD-like proteases. These putative pilins are present only in the euryarchaeota, and they appear to be processed by a specialized class III signal peptidase, EppA (75). EppA is a substantially larger protein than FlaK, as it contains four additional predicted transmembrane domains.

Very recently, several reports have appeared that, for the first time, address the structure, function, and genetics of pili in a variety of archaea, including *Methanococcus*, *Sulfolobus*, and *Methanothermobacter*. In *Methanococcus maripaludis*, a *flaK* gene deletion mutant was constructed (S. Ng et al., unpublished data). This mutant is nonmotile and nonflagellated; observations by electron microscopy revealed that this strain possessed pili as the sole cell surface appendage (Fig. 6). The pili were isolated at high purity following detergent extraction of cells and polyethylene glycol precipitation. Subsequent sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis revealed the presence of a single major band of ~17 kDa that was presumed to be the major pilin protein. Mass spectrometry analysis of the pilus preparation identified one of the products (MMP0236) of the putative gene cluster identified earlier (75). In-frame deletion of the putative major pilin gene, MMP0236, rendered the cells nonpiliated. Complementation led to a restoration of piliation, providing direct genetic evidence linking the gene to this novel structure (Ng et al., unpublished data).

An interesting recent finding linked the glycosylation pathway to the assembly of both flagella and pili in *M. maripaludis*. Deletion of an acetyltransferase gene involved in the biosynthesis of the glycan that is N linked to the flagellins resulted in nonflagellated cells. Unexpectedly, this mutant also lacked pili on the cell surface. Pili were found in the culture supernatant,

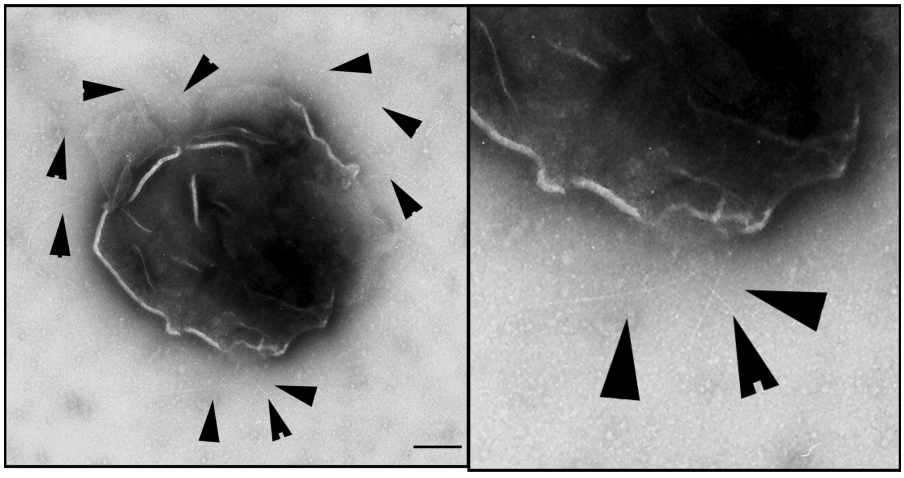

FIG. 6. Pili on the surface of a *flaK* mutant of *Methanococcus maripaludis*. This mutant cannot make flagella, leaving the peritrichously located pili evident as the only surface structure remaining on the cell surface. The sample was negatively stained with 2% phosphotungstic acid, pH 7. Bar, 200 nm. Courtesy of S.-I. Aizawa, reproduced with permission.

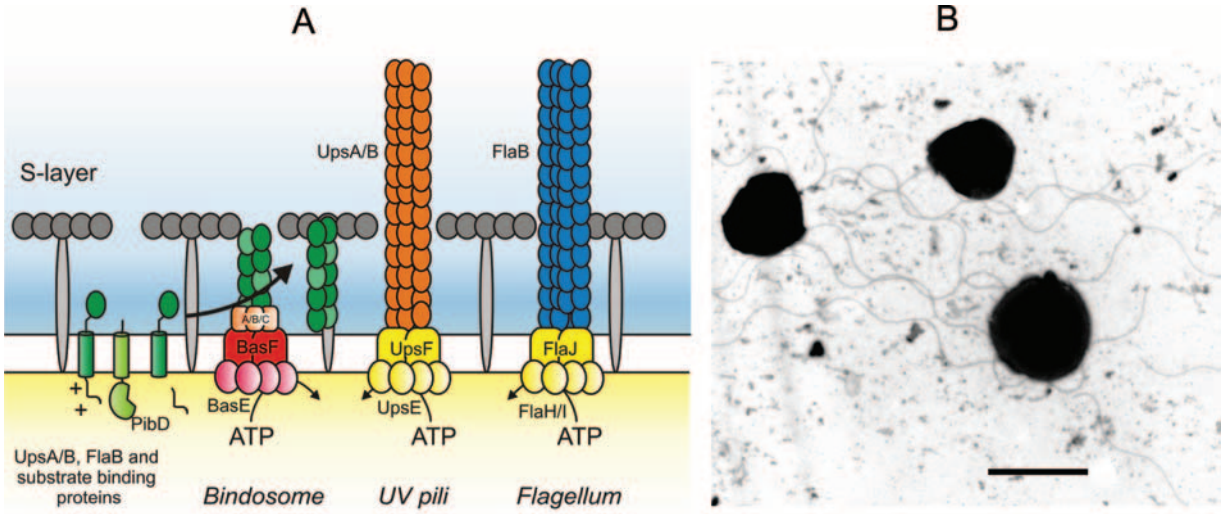

FIG. 7. (A) Model of the assembly of surface structures in the cell envelope of *Sulfolobus solfataricus*: Precursor proteins (SBPs, prepilins, or preflagellins) are processed by PibD and are then inserted by their specific assembly system either in the bindosome structure, the UV inducible pili or the flagellum. The exact nature of the bindosome structure is not known yet, and an alternate format, shown attached to the S-layer, is also indicated. All three assembly systems share the same core of the machinery: an integral membrane protein and a cytoplasmic ATPase. (B) Electron micrograph of flagellated *S. solfataricus* P2 cells. Flagella are present all around the cells and do not appear bundled. Bar, 1 μm.

indicating that deletion of the gene did not affect pilus formation per se but rather affected attachment of the pili to the cell surface (82).

Recent structural studies on the *M. maripaludis* pili indicated that even though the pilins had similarities to bacterial type IV pilins, the structure formed by the archaeal pilins was unlike that seen in any of the known bacterial pili (86). Two subunit packing arrangements were found, and both were found to coexist within the same filaments. In addition, the pili contain a central lumen only slightly smaller than that observed in bacterial flagella and type III secretion system needle structures that grow by addition of subunits at the distal end.

A recent study on UV stress in *Sulfolobus* showed that *Sulfolobus* cells start to aggregate and form pili upon treatment with UV light (31). An operon containing possible pilin proteins, SSO0117/118, and a secretion ATPase, SSO0120, was highly induced (1, 31). A deletion mutant of the assembly ATPase SSO0120 rendered cells unable to form pili and aggregate after UV stress (S. Froels et al., unpublished data). The pili are thought to play a role in cell aggregation prior to conjugation (Fig. 7).

In *Methanothermobacter thermoautotrophicus*, pili were isolated and shown to be composed of the *mth60* gene product. Antibodies raised to the pilin protein produced in *Escherichia coli* reacted with the 16-kDa pilin glycoprotein and could be used to detach adhering *M. thermoautotrophicus* cells from surfaces. Cells grown on surfaces had many more pili than cells grown in liquid culture. This study demonstrated yet another role for pili in archaea, namely, that of adhesion (79).

Investigation of the biochemistry, genetics, and functions of archaeal pili has only recently begun. Consequently, it is presently unclear if archaea possess the vast diversity of pilus types, with assorted functions and assembly mechanisms, presently known to occur in bacteria.

BINDOSOME

An interesting proposed archaeal structure of unique function is the bindosome in *S. solfataricus* (2). So far, the actual structure has not been visualized in the membranes of *S. solfataricus*, but rather it is thought to be a pilus-like structure close to the cytoplasmic membrane or integrated within the S-layer (Fig. 7). The main evidence in support of the presence of this hypothesized structure is that the proposed structural components, the substrate binding proteins (SBPs), contain class III signal peptide sequences, a feature typical of proteins which are well known to form oligomeric structures in both archaea and bacteria. The oligomerized complex is proposed to play a role in facilitating sugar uptake, a function that enables *S. solfataricus* to grow on a broad variety of substrates.

Sulfolobus species are hyperthermophilic acidophiles typically found in volcanic springs, with optimal growth at around pH 2 to 3 in the temperature range of 75 to 80°C. One interesting distinction that draws *S. solfataricus* apart from other *Sulfolobus* species, such as *S. tokodaii* and *S. acidocaldarius*, is the ability to grow on a wide variety of sugars as its only carbon source. Previous studies showed that *S. solfataricus* has a wide range of ABC transport systems for sugar uptake (4, 26). ABC transporters encompass the actual transport domain in the membrane and cytoplasmically located ATPases, which drive the transport of the substrate by ATP hydrolysis. At the periplasmic side of the membrane, SBPs bind the substrate and deliver it to the transport domain. Interestingly, the signal peptides from one class of sugar binding proteins of *S. solfataricus* resemble class III signal peptides found in archaeal flagellins or bacterial pilins. In vitro assays demonstrated that precursors of these binding proteins are indeed processed by the class III signal peptidase, PibD, of *S. solfataricus* (3). In contrast to FlaK, PibD showed a quite broad substrate specificity, as it is able to process not only the SBPs but also the

flagellin and pilin SSO0118 of *S. solfataricus* (3; S. Albers, unpublished data).

Deletion mutant studies identified an assembly system that is required for the correct cell surface localization of the SBPs (88). The Bas (*b*indosome *a*ssembly *s*ystem) operon contains five genes: *basABC*, three small genes that encode pilin-like proteins with class III signal peptides; *basE*, encoding an ATPase; and *basF*, encoding an integral membrane protein. BasE and -F are homologues of TadA and -C of *Aggregatibacter* (*Actinobacillus*) *actinomycetemcomitans*, respectively, which are involved in the assembly and export of Flp pili, which are needed for tight adherence (40). Analysis of *S. solfataricus* mutants showed that the deletion of *basABC* only moderately affected growth on rich media, whereas the Δ*basEF* strains showed a profound decrease in growth rate. However, growth on minimal sugar media containing sugars (arabinose or glucose) that are transported by SBPs with class III signal peptides was abolished in the Δ*basEF* strain, while only slight growth was apparent in the Δ*basABC* strain. Growth on a sugar (maltose) that enters the cell via an SBP with a class I signal peptide was normal for both knockout strains (88). Although in these deletion strains the glucose binding protein was inserted into the membrane and active in substrate binding, no glucose uptake could be observed. This suggested that the Bas system is essential for the correct surface localization of the SBPs to enable them to function in substrate uptake. BasABC might regulate the assembly of the binding proteins by a mechanism similar to that of the assembly of pseudopili that are functional domains of type II secretion systems, whereas BasEF may form the core of the assembly machinery in the membrane (88). A program designed to identify proteins with class III signal peptides (FlaFind) (75) predicted the presence of SBP precursors in many sequenced archaeal genomes; *S. solfataricus* contains eight such proteins, whereas most other archaea seem to contain only one predicted SBP with a class III signal peptide. *Methanosarcina acetivorans* harbors six predicted SBPs (75). This suggests that the bindosome is more widely distributed among archaea.

The controlled assembly/surface localization of SBPs probably enables the organism to scavenge nutrients more readily from the environment to concentrate substrates in the periplasmic space between the S-layer and the cytoplasmic membrane. The bindosome may represent an affinity cascade that channels substrates to the ABC transporters. Whether the SBPs are assembled into a pilus-like structure or are attached in a controlled manner to the S-layer remains to be determined (Fig. 7).

CONCLUDING REMARKS AND OUTLOOK

While the study of archaeal cell surface structures is fascinating in its own right, its scope extends far beyond that and has enriched our understanding of many processes central to archaeal cellular functions. The archaeal flagellum has been the prototype for a specialized secretion system in the domain *Archaea*. Its study has broadened our understanding of archaeal signal peptides and the archaeal preflagellin peptidase, an enzyme with an amalgam of bacterium-like and archaeon-specific traits (56). The bindosome structure in *S. solfataricus* is a proposed unique case that has adopted the utilization of the archaeal flagellum/bacterial type IV pilus mode of secretion/assembly for an entirely different type of cell surface organelle involved in sugar uptake.

The recent expansion of information on the archaeal pili has generated much excitement; their possible cellular function is an area of active research in several diverse archaeal species. While the specificity and exclusivity of the prepilin peptidase-like enzymes in the flagellum system versus the pilus system have been demonstrated (75), the proposed commonalities in the assembly of the two surface structures make it tempting to speculate that some of the accessory proteins could be shared. Studies need to be undertaken to further characterize the two systems, independently of each other as well as addressing possible interrelatedness.

Hami and cannulae are archaeon-specific cell surface structures that represent unique adaptations to the environment that are not observed on bacterial cells. The assembly mechanisms of such unusual structures are expected to be novel and to contribute new information and strategies on how surface-localized structures may be assembled in prokaryotes. Unraveling of their mysteries will be dependent upon advances in understanding the genetics of these organisms.

Due to the increasing availability of improved genetic tools for many archaea, we have witnessed an expansion of knowledge in archaeal research in general and on archaeal cell surface structures in particular. In comparison to the wealth of information available about bacterial surface structures, our current knowledge on archaeal counterparts is just the tip of an iceberg. Continued effort is needed to yield a better understanding of the assembly and functions of the fascinating structures discovered to date, as well as to discover and characterize the ones yet unknown to us.

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